Appl. No.	:	09/250,056	Confirmation No. 1647
Applicant	:	James D. Marks, et al.	
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MAIL STOP AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132
[M.P.E.P. § 715.01(c)]

I. I, Dmitri B. Kirpotin, am Vice President, Pharmaceutical Research and Development at Hermes Biosciences, Inc., South San Francisco, CA. Accompanying this Declaration is a copy of my Biographical Sketch. As should be apparent from this information, I am an expert in the field of immunology, particularly as it relates to cancer/tumor-related antigens and antibodies. I have read the Office Action dated December 31, 2003 and the above-identified application.

II. The epitopes bound by the antibodies designated as F5 and C1 in US Patent Application No. 09/250,056 are not the same epitopes that are bound by the antibodies reported by Maier et al. (1991), Xu et al. (1993), and Shawner et al. (1994).

III. Cellular receptor molecules modulate physiological and molecular processes in a living cell. Binding of an antibody to a selected epitope on a cell surface receptor molecule of a cancer cell is capable of eliciting certain physiological responses. A person skilled in the art of cancer cell-specific antibodies would recognize that the nature and the degree of the physiological response is related to, and is indicative of, a particular epitope to which the antibody specifically binds. Thus, finding of a dramatic difference in the nature or magnitude of the cellular physiological response elicited by two antibodies would lead a skilled artisan to conclude that the two antibodies bind to different epitopes.

IV. The proto-oncogene c-ErbB-2 (also termed *HER2*) encodes a 185 kD tyrosine kinase receptor of the EGF receptor family. While the nature or function of the natural ligand of c-ErbB-2 remain uncertain, it is known that the molecules of c-ErbB-2, as part of their receptor activity, a) associate with each other as well as with the other members of EGFR family, b) undergo phosphorylation of their intracellular domains, c) evoke a cascade of intracellular processes leading, e.g., to the modulation of cell growth, and d) undergo internalization into the cell. A number of murine monoclonal antibodies in the art have been shown to bind to the ErbB2 receptor and induce the above-described cellular responses to various degrees. It was noted, for example, by Maier, et al. (1991) that antibodies that cause a cellular response of similar nature and magnitude are likely to bind to the same epitope. Accordingly, antibody binding to different epitopes within the receptor structure often leads to differing cellular responses to such binding (i.e., dissimilar rates of internalization, or effectiveness as a growth inhibitor). Furthermore, binding of different antibodies to the same epitope can be demonstrated using a competition assay.

V. F5 and C1 antibodies recognize the same epitope. Binding of the F5 and/or C1 epitope of the c-ErbB-2 receptor by the antibody sequences of the claimed invention leads to extremely rapid internalization of the antibody into (c-ErbB-2)-expressing cancer cells, such as SKBr-3 cells (Example 2, starting at page 61). Cellular binding of phage expressing F5, C1 or C6.5 sequences was inhibited in a competition assay by increasing concentrations of soluble scFv-F5, scFv-C1 or

scFv-C6.5 (see, for example, Figure 2 in the specification). Cells exposed to the F5 or C1 antibody have been shown to internalize about 70-80% of the cell-bound antibody over the period of 5-6 hours (See Neve (2001)"Biological effects of anti-ErbB2 single chain antibodies selected for internalizing function" Biochem. Biophys. Res. Comm. 280:274-279). Thus, one of skill in the art would surmise that the two antibody sequences recognize similar epitopes, based upon the demonstrated cross-reactivity in the competition studies as well as similarities in dissociation constants and internalization rates (page 69, lines 14-28 of the specification).

VI. The same competition assay was used to confirm that F5 and C1 recognize a different epitope than the Genentech anti-ErbB2 antibody 4D5 (which, when humanized, is referred to as Herceptin). Furthermore, the 4D5-related antibodies do not recognize the F5 or C1 epitopes. The Neve et al. 2001 publication (*supra*) provides a direct comparison between F5 internalization and the internalization of the whole IgG 4D5 and Fab fragment of 4D5 (see Figures 1-4). Thus, antibodies that are cross-reactive with 4D5-related constructs must necessarily recognize a different epitope than that of the F5 and C1 antibodies.

VII. The data by Maier et al., 1991, lead a skilled artisan to conclude that antibodies described therein do not recognize the F5- or C1-binding epitopes. The Maier reference (Cancer res. 51:5361-5369,1991) cites the immunoglobulin TA1. Only 12% of this antibody is internalized after up to 4 hours incubation at 37°C with a rat cell line artificially transfected with HER2 receptor (see the HER2-positive test cell lines in Fig. 3, page 5364). A subsequent publication from the same research group (Boyer, et al. (1999) Int. J. Cancer, 82, 525-531) cited internalization data for TA1 and other antibodies into SKBr-3 cells. The percentage internalization for TA-1 and related conjugates ranged from 7% to 13% (Table IV on page 259). This value is dramatically lower than the 70-80% internalization rates of the antibodies provided in the subject invention. Thus, one of skill in the art would surmise that the Maier antibodies recognize a different epitope from that recognized by F5 and C1.

VIII. It is known that generation of a monovalent antibody species, such as single chain Fv, from a multivalent one, such as an immunoglobulin, reduces the binding affinity and the magnitude of a physiological response (for example, cell growth inhibition). Thus, one of skill in the art would also conclude that generation of single chain antibody sequences from the poorly-internalized antibodies provided in Maier is highly unlikely to produce the well-internalized antibodies described in USSN 09/250,056.

IX. The data of Xu et al, 1993, lead a skilled artisan to conclude that the antibodies described therein do not recognize the F5- or C1-binding epitopes. The Xu reference (1993 Int. J. Cancer 53: 401-408) cites 11 antibodies reactive against HER2, for which the internalization data (into SKBr-3 cells) was presented for two members, TA1 and ID5. The internalization efficiency determined for TA1 is 30% after 1 hour, and for ID5 is 27% after one hour. While the data was not shown, Xu indicated that the other antibodies examined in the studies were internalized at similar rates despite their differing abilities to inhibit cell growth (page 406, second column). Thus, one of skill in the art would surmise that the Xu antibodies recognize a different epitope from that recognized by F5 and C1. Furthermore, one of skill in the art would not expect to generate rapidly internalized single chain antibody sequences using the poorly-internalized antibodies provided in Xu et al.

X. The data of Shawver et al., 1994, lead a skilled artisan to conclude that the antibodies described therein do not recognize the F5- or C1-binding epitopes. In the final publication cited by the Examiner (Shawver et al. 1994 Cancer Res. 54: 1367-1373), five antibodies are described: Tab 250, Tab 255, Tab 257, Tab 260, and Tab 263. These antibodies differed in their growth inhibiting capacity, Tab 250 having the strongest inhibitory activity , and TAb 263 being the weakest inhibitory activity. The rate of internalization of the TAb antibodies into SK-Br-3 cells was determined to be 28% at 3 hours (the maximum duration of the experiment; see p. 1369, last paragraph, and p. 1371, Fig. 6). Thus, one of skill in the art would surmise that the Shawver antibodies recognize a different epitope from that recognized by F5 and C1. Furthermore, one of skill in the art would not

expect to generate rapidly internalized single chain antibody sequences using the poorly-internalized antibodies provided in Xu et al.

XI. In summary, the various erbB2-binding antibodies provided by Maier, Xu and Shawver have SKBr-3 internalization rates ranging between 7% and 30% (measured at 37°C for between 1-4 hours). One of skill in the art would reasonably surmise that any single chain antibodies generated using these sequences would bind to the same epitope as the parent antibody and have similarly low (or lower) internalization rates. The antibodies provided in USSN 09/250,056 (i.e., antibodies which recognize the F5- or C1-binding epitope) lead to surprisingly efficient (70-80%) internalization in SKBr-3 cells under similar experimental conditions. Given that receptor internalization is a type of cellular response consequential to the interaction between the receptor and a ligand (such as a receptor-specific antibody), a skilled artisan would conclude that, since the internalization rates differ so dramatically between the antibodies cited in the publications and the antibodies of the subject application, the antibodies disclosed in the prior art do not bind to the F5 or C1 epitope.

XII. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Declarant's signature:

Dmitri B. Kirpotin, Ph.D.

04/29/2004

Date

BIOGRAPHICAL SKETCH

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NAME	POSITION TITLE
Dmitri B. Kirpotin	Vice President, Pharmaceutical Research and Development

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Moscow State University, Moscow, Russia	M.S. equiv.	1977	Chemistry
A.N.Bach Institute of Biochemistry, Moscow, Russia	Ph.D. equiv.	1983	Biochemistry
University of Colorado Cancer Center, Denver, CO, USA	postdoctoral	1990-1995	Medical Oncology

A. Positions and Honors.

Positions and Employment

1977-79	Professional Research Assistant, A.N.Bach Institute of Biochemistry, Moscow, USSR
1979-82	Predoctoral Fellow, Ph.D. Program in Biochemistry, A.N.Bach Institute of Biochemistry, Moscow, USSR
1983-90	Research Scientist, Senior Research Scientist, A.N.Bach Institute of Biochemistry, Moscow, USSR
1991	Visiting Scientist, Max-Planck Research Unit for Immunology\Rheumatology, Erlangen, Germany
1991	Postdoctoral Fellow, Department of Cell and Developmental Biology, University of California at Davis, Davis, CA
1990-95	Postdoctoral Fellow, Research Fellow, Division of Medical Oncology, University of Colorado Health Sciences Center, Denver, CO
1995-2002	Assistant Research Biochemist, Dept. of Cellular and Molecular Pharmacology, Dept. of Radiation Oncology, Dept. of Medicine, University of California San Francisco, San Francisco., CA
1998-2003	Associate Scientist, Scientist, California Pacific Med. Center Research Institute, San Francisco, CA.
1998-present	Vice President, Research Discovery, Pharmaceutical Research and Development, Hermes Biosciences, Inc., South San Francisco, CA

Other Experience and Professional Memberships

1991	American Association for Cancer Research, associate member; member (1997).
1993	University of Colorado Cancer Center, member
1996	American Society of Cell Biology, member .
1997	Radiation Research Society, North American Hyperthermia Society, member.
1998	American Association of Pharmaceutical Scientists, member
2000	UCSF Comprehensive Cancer Center, member
2003	American Association for Advancement of Science, member

Honors

1973-76	Academic excellence scholarship, Moscow State University
1977	Honors Diploma, Moscow State University.
1985,1987	The Creative Youth in Science and Technology, USSR National Fair, Bronze Award,
1991	Deutsche Academische Austaushdienst, fellowship (Max-Planck-Society)
1993-1995	University of Colorado Cancer Center, SPORE in Lung Cancer, Career Development award
1994	University of Colorado School of Medicine, Thorkildsen Medical Research Fellowship,

Patents

6 issued, 5 pending US patents.

Administrative/Organizational

CPMCRI IACUC member

B. Selected publications (in chronological order).

(Publications selected from approximately 70 peer-reviewed publications).

1. D.B.Kirpotin, S.D.Wolfson, S.V.Obukh. (1987) Comparative analysis of methods for the blockade of mononuclear phagocyte system. - *Immunology (Moscow)*, 1987(6):76-77.
2. S.D.Wolfson, D.B.Kirpotin. (1987) The use of liposomes for the indication of the functional state of mononuclear phagocyte system. - *J. Microbiology, Epidemiology and Immunobiology (Moscow)*, 1987(11):73-76
3. D.B.Kirpotin, A.F.Orlovsky, Yu.A.Motorin, K.L.Gladilin. (1987) The use of insoluble polyelectrolyte complexes for the preparation of magnetic drug microspheres. - In: *Magnetic Hydrodynamics, vol. 4.* Salaspilss, Latvija, IF AN LatvSSR, p.83-86.
4. D.B.Kirpotin, A.F.Orlovsky, K.L.Gladilin. (1989) Interactions of ionic surfactants with the water-insoluble polyelectrolyte complex. - In: *Interpolymer Complexes.*Riga, Latvija, Latv.AN, p.121-123.
5. D.B.Kirpotin, R.Kinne, A.Milton, E. Palombo-Kinne, F.Emmrich. (1993) Magnetic targeting of a therapeutic antibody using magnetotropic microspheres of the interpolyelectrolyre coacervation complex. - *J. Magn. Magn. Materials,* 122:354-359
6. D.C.F.Chan, D.B.Kirpotin, P.A.Bunn, Jr.(1993) Synthesis and evaluation of colloidal magnetic iron oxides for the site-specific radiofrequency-induced hyperthermia of cancer. - *J. Magn. Magn. Materials*,122:374-378
7. D.Kirpotin, K.Hong, N. Mullah, D.Papahadjopoulos, S.Zalipsky. (1996) Liposomes with detachable polymer coating: destabilization and fusion of dioleoylphosphatidylethanolamine vesicles triggered by cleavage of surface-grafted poly(ethylene glycol). - *FEBS Lett.*, 388:115-118.
8. D.Kirpotin, J.W. Park, K.Hong, S.Zalipsky, W.-L.Li, P.Carter, C.C. Benz, D.Papahadjopoulos (1997) Sterically stabilized anti-HER2 immunoliposomes: desin and targeting to human breast cancer cells *in vitro*. - *Biochemistry* 36:66-75.
9. J.W.Park, K.Hong, D.Kirpotin, D.Papahadjopoulos, C.Benz (1997). Immunoliposomes for cancer treatment. *Adv. Pharmacology* 40, 399-436.
10. J.W.Park, K.Hong, D.Kirpotin, O.Meyer, D.Papahadjopoulos, C.Benz (1997). Anti-HER2 immunoliposomes for targeted therapy of human tumors. *Cancer Letters* 118:1-8.
11. D.Chan, D. Kirpotin, P.A.Bunn,Jr. (1997). Physical chemistry and in vivo tissue heating properties of colloidal magnetic iron oxides with increased power absorption rates. - In: *Scientific and Clinical Aspects of Magnetic Carriers*/ Ed. by U. Hasely, W. Schutt, J.Teller, M. Zborossky. Plenum Press, N.Y. p.607-618.
12. D.Kirpotin, J.W.Park, K. Hong, Y.Shao, R.Shalaby, G.Colbern, C.C.Benz, D. Papahadjopoulos (1997) Targeting of liposomes to solid tumors: the case of sterically stabilized anti-HER2 immunoliposomes. - *J. Liposome Res.* 7:391-417.
13. D.Kirpotin, J.W.Park, K.Hong, Y.Shao, G.Colbern, W-W.Zheng, O.Meyer, C.C.Benz, D.Papahadjopoulos (1998) Targeting of sterically stabilized liposomes to cancers overexpressing HER2/neu proto-oncogene. - In: *Medical Applications of Liposomes*/ Ed. by D.Lasic, D.Papahadjoloulos. Elsevier, Amsterdam, p.325-345.

14. O.Meyer, D.Kirpotin, K.Hong, B.Sternberg, J.W.Park, M.W.Woodle, D.Papahadjopoulos (1998) Cationic liposomes coated with poly(ethylene glycol) as carriers for antisense oligonucleotides. - *J. Biol. Chem.*, 273:15621-15627.
15. J.W.Park, D.Kirpotin, K.Hong, W.Zheng, Y.Shao, O.Meyer, C.C.Benz, D.Papahadjopoulos (1998) Sterically stabilized immunoliposomes: formulations for delivery of drugs and genes to tumor cells *in vivo*. In*: Targeting of Drugs. 6. Strategies for Stealth Therapeutic Systems.* /Ed. by.Gregoriadis and McCormack. Plenum Press, N.Y., p. 41-47
16. J.W.Park, D.Kirpotin, K.Hong, G.Colbern, R.Shalaby, Y.Shao, O.Meyer, U.Nielsen, J.Marks, C.C.Benz, D.Papahadjopoulos (1998). Anti-HER2 immunoliposomes for targeted drug delivery. *Med. Chem. Res.* 8:383-391
17. D. Papahadjopoulos, D. Kirpotin, J. W. Park, K. Hong, Y. Shao, R. Shalaby, G. Colbern, and C. C. Benz(1998) Targeting of drugs to solid tumors using anti-HER2 immunoliposomes. *J. Liposome Research* 8:425-442.
18. D.C. Drummond, O.Meyer, K.Hong, D.Kirpotin, D. Papahadjopoulos (1999). Optimizing liposomes for delivery of anti-cancer agents to solid tumors. *Pharm. Rev.* 51:691-743.
19. K. Hong, D.B. Kirpotin, J.W. Park, Y.Shao, R.Shalaby, G.Colbern, C.C. Benz, and D. Papahadjopoulos (1999) Anti-HER2 immunoliposomes for targeted drug delivery. *Ann. N. Y. Acad. Sci.* 886: 293-296.
20. W.C.Krauss, J.W.Park, D.B.Kirpotin, K.Hong, C.C.Benz (2000) Emerging antibody-based HER2(ErbB2/neu) therapeutics. *Breast Disease* 11: 113-124.
21. D.C. Drummond, K.Hong, J.W.Park, C.C.benz, D.B.Kirpotin (2001). Liposome targeting to tumors using vitamin and growth factor receptors. In: *Vitamins and Hormones* /Ed. by G. Litwack. vol. 60. Acad. Press, N.Y., p.286-332.
22. R.M. Neve, U.B. Nielsen, D.B. Kirpotin, M.-A. Poul, J.D. Marks, C.C. Benz (2001) Biological effects of anti-ErbB2 single chain antibodies selected for internalizing function. *Biochem. Biophys. Res. Commun.* 280:274-279.
23. J. W. Park, D. B. Kirpotin, K. Hong, R. Shalaby, Y. Shao, U. B. Nielsen, J. D. Marks, D. Papahadjopoulos, C. C. Benz (2001). Tumor targeting using anti-her2 immunoliposomes. *J Controlled Release* 74:95-113.
24. J.W.Park, K.Hong, D.B.Kirpotin, G.Colbern, R.Shalaby, J.Baselga, Y.Shao, U.Nielsen, J.Marks, D.Moore, D.Papahadjopoulos, and C.C.Benz.(2002) Anti-HER2 immunoliposomes: enhanced anticancer efficacy due to targeted delivery. *Clinical Cancer Res. 8:1172-1181*
25. U.B.Nielsen, D.B.Kirpotin, E.M.Pickering, K.Hong, J.W.Park, M.R.Shalaby, Y. Shao, C.C.Benz, J.D.Marks. (2002) Therapeutic efficacy of anti-ErbB2 immunoliposomes targeted by a phage antibody selected for cellular internalization. *Biochim. Biophys. Acta* 1591:109-118.
26. C. Mamot, D.C. Drummond, U. Greiser, K. Hong, D.B. Kirpotin, J.D. Marks, J.W. Park. (2003) Epidermal growth factor receptor (EGFR)-tageted immunoliposomes mediate specific and efficient drug delivery to EGFR- and EGFRvIII-overexpressing tumor cells. *Cancer Res.* 2003, 63: 3154-3161.
27. C. Mamot, D.C.Drummond, K. Hong, D.B. Kirpotin, J.W.Park. (2003) Liposome-based approaches to overcome anticancer drug resistance. *Drug Resist. Update* 6: 271-279.
28. B. Liu, F. Conrad, M.R.Cooperberg, D.B. Kirpotin, J.D.Marks. (2004) Mapping tumor epitope space by direct selection of single-chain Fv antibody libraries on prostate cancer cells. *Cancer Res.* 64: 704-710.
29. R. Saito, J.R. Bringas, T.R. McKnight, M.F. Wendland, C. Mamot, D.C.Drummond, D.B. Kirpotin, J.W.Park, M.S.Berger, K.S.Bankiewicz (2004). Distribution of liposomes into brain and rat brain tumor models by convection-enhanced delivery monitored with magnetic resonance imaging. *Cancer Res.* 64:2571-2579.